FOLIAGE AND WINTER WOODY BROWSE QUALITY OF AN IMPORTANT *SALIX* BROWSE SPECIES: EFFECTS OF PRESENCE OF ALDER-DERIVED NITROGEN AND WINTER BROWSING BY ALASKAN MOOSE (*ALCES ALCES GIGAS*)

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Abstract

In this study, I examined the relationship between soil nitrogen and winter browsing by moose on the physical and chemical characteristics of *Salix alaxensis*; specifically stem production, leaf nutritional quality, and stem nutritional quality of tissues produced the following growing season. I measured stem biomass production the 2013 growing season and offtake during the 2013-2014 winter browsing season at 16 sites on the Tanana River floodplain near Fairbanks, Alaska. I revisited the sites the following summer and autumn to assess regrowth and to collect soil, foliage, and stem samples. Browsing intensity and total soil nitrogen were similar in sites with and without alder, a nitrogen-fixing shrub. Soil nitrogen and browsing intensity were not consistently related to changes in stem or leaf quality, although there were significant relationships in some subsets. Soil nitrogen and browsing intensity also did not have consistent relationships with stem regrowth the following growing season. These results indicate that *S. alaxensis* growing in this system are able to recover from a naturally broad range of browsing utilization, including very high levels of offtake, and continue to produce nutritious leaves and stems.

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GENERAL INTRODUCTION

Herbivory can drive changes in many physical and chemical plant characteristics, including phenotype, nutritional quality, toxin production, and the ability of plants to produce reproductive tissue (Zasada et al. 1987, Stamp 2003, Veraart et al. 2006). Changes can be short-lived or persistent, and can occur in existing tissues or new tissues produced after the herbivory occurs (Bryant 1991, Bryant 2003). These changes can improve or reduce the attractiveness of plants as forage by altering the ability of herbivores to ingest and/or digest plant materials. For instance, winter browsing of feltleaf willow (*Salix alaxensis*) stems increases the nitrogen concentration and reduces the tannin activity in feltleaf willow leaves the next growing season, making those leaves more nutritious (Bryant 2003). Conversely, leaf damage caused by willow leaf blotch miners decreases the stem biomass available for moose the following winter (Allman et al. 2018).

Stem production by woody plants often changes in response to vertebrate browsing, but the nature of that change is dependent a variety of factors. The response of stem production to browsing events is dependent on the plant species, the proportion of biomass removed, browsing history, the season in which browsing occurs, and the productivity of the ecosystem (Bergstrom and Danell 1987, Danell et al. 1994, Persson et al. 2007, Den Herder et al. 2009). In some cases, browsing reduces the total aboveground biomass produced by a plant, especially if the browsing level is high (Persson et al. 2007). Alaskan feltleaf willows are known to respond to winter browsing by producing fewer and larger stems, and repeated browsing can lead to a "broomed" growth form with multiple competing apical stems on a ramet (Seaton 2002, Bowyer and Neville 2003). In the long term, sustained high offtake by browsing ungulates can negatively impact woody plants in a variety of ways. Browsing on dormant stems can influence the ability of the

plants to regrow, survive, and successfully flower, which can result in changes to the plant population age structure, and even the plant communities, as less palatable species outcompete those that are utilized by browsing herbivores (Peinetti et al. 2001, Butler and Kielland 2008).

The production of carbon-based plant defenses is influenced by the carbon to nitrogen balance within a plant (Bryant et al. 1983, Bryant 1987, Bryant et al. 1987, Ayres et al. 2004). Increased soil nitrogen availability tends to decrease carbon-based plant defenses by redirecting primary plant metabolites to growth rather than defensive compounds (Bryant et al. 1987). Nitrogen fixation by alder is a major input of nitrogen into many interior Alaskan ecosystems (Nossov et al. 2011), and alders are an indirect supply of nitrogen to other plant species through the decomposition of alder tissues. Increased soil nitrogen has been hypothesized to be a driving factor in succession, by making preferred forage species more palatable and hastening the establishment of less desirable later-successional species (Bryant 1987). Whereas the effects of commercial nitrogen fertilization on plant defenses have been studied in depth in the family *Salicaceae*, the influence of natural fertilization by nitrogen fixers has not been well documented in the field (Bryant 1987, Bryant et al. 1987, Albrectsen et al. 2004), though in principle the effect may be quite similar.

In the Alaskan boreal forest, the genus *Salix* is an important component of moose winter browse (Densmore et al. 1987, Risenhoover 1989). In Alaska, *Salix alaxensis* (feltleaf willow) is one of the most important browse species, and its stems represent approximately 50% of the total biomass consumed by the regions' wintering moose (Risenhoover 1989). Moose prefer feltleaf willow stems to balsam poplar, quaking aspen, and several other willow species due to its high digestibility and protein concentration (Wolff and Zasada 1979, Kielland and Osborne 1998). However, similarly to many other *Salix* species, feltleaf willow produces condensed tannins at

levels that can significantly reduce protein availability, and this production may be influenced by the intensity of herbivory (Bryant et al. 1985, Bryant 1987, Collins 2002).

The Game Management Units (GMUs) surrounding Fairbanks are intensively managed by the Alaska Department of Fish and Game (ADFG) for increased hunting opportunities. Moose populations in these units, especially the Tanana Flats (GMU 20A), have been high (over 2 moose/km^2) since the mid 1990's (ADFG 2011). The large moose population causes high levels of browsing on preferred forage species in this GMU (Butler and Kielland 2008) which has recently been manifested in negative demographic effects on winter survival, twinning rates, and calf survival of moose (Seaton et al. 2011). How this prolonged browsing pressure has affected foliage and stem quality of feltleaf willows in the region is uncertain.

The high density of moose along the Tanana River and their preferential use and dependence on a few forage species makes the Tanana river floodplain an excellent study system for plant/herbivore interactions. The presence of alder stands of varying densities is also ideal for studying the effects of naturally fixed nitrogen on plant defensive chemistry. Considering these observations, I sought to examine two questions:

1) How does winter browsing by moose affect the regrowth and chemistry of feltleaf willows?
2) How does the presence of alder-derived nitrogen in the soil modify those responses?

To address these questions, I conducted observational field studies along the Tanana River floodplain between Fairbanks and Nenana to examine the relationships between winter browsing by moose and alder-derived soil nitrogen concentration, with the regrowth, nitrogen concentration, and biological activity of condensed tannins in current annual growth (CAG)

tissues of feltleaf willow. My general hypothesis was that the range of natural browse utilization by moose between study sites influences the regrowth and chemistry of feltleaf willows, and that changes in soil nitrogen concentration alter that effect by shifting carbon between defense and growth.

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CHAPTER 1: FOLIAGE AND WINTER WOODY BROWSE QUALITY OF AN IMPORTANT *SALIX* BROWSE SPECIES: EFFECTS OF PRESENCE OF ALDER-DERIVED NITROGEN AND WINTER BROWSING BY ALASKAN MOOSE (*ALCES ALCES GIGAS*)

ABSTRACT: Plant chemistry and growth can be altered by browsing by herbivores. Changes can be immediate and highly specific, as in chemical reaction to herbivore salivary proteins, or long-term and more general, through changes in meristem competition or plant carbon:nitrogen ratios. These changes can alter the quantity and nutritional quality of plant tissues both within current year tissues and those produced during following growing seasons. The effects of winter browsing on plant tissues produced the following growing season are largely unknown, but may be important for the management of ungulates, such as moose. In this study, I examined the relationships between natural winter stem browsing by moose on the regrowth and chemical composition of leaves and stems produced by feltleaf willows the following growing season. These relationships were examined in willow stands in the presence and absence of alder, a nitrogen fixing shrub which may modulate the nitrogen economy of willows. Browsing had variable relationships with regrowth and tannin activity, and these effects varied in the presence and absence of alders. Whereas browse consumption was proportional to browse production, relative regrowth after browsing showed no response to the 3-fold variation in offtake. However, increased offtake increased plant quality via reduced tannin activity, and the presence of alders increased foliar nitrogen concentrations. These results suggest that feltleaf willows growing on[1]

[1] BURROWS, J., and KIELLAND, K. Foliage and winter woody browse quality of an important *Salix* browse species: effects of presence of alder-derived nitrogen and winter browsing by Alaskan moose (*Alces alces gigas*). Prepared for submission to Alces.

interior Alaskan floodplains are highly resilient to browsing and can maintain the production of nutritious biomass across a wide range of browsing pressure.

INTRODUCTION

Browsing can cause many changes to plant chemistry and morphology, including altered growth rate, chemical defenses, growth form, and reproductive success (Bergstrom and Danell 1987, Bryant 1991, Persson et al. 2007). The response to browsing depends on a variety of factors, including the species of plant being browsed, the season browsing occurs, the ontogenetic stage of the plant, herbivore species, and the tissue being browsed (Chapin et al. 1985, Keefover-Ring et al. 2016, Allman et al. 2018). The responses can be rapid or delayed, and may be detected in existing tissues, or those produced in future seasons (Fields and Orians 2006, Allman et al. 2018).

Compared to browsing on leaves, the effects of vertebrate browsing on stems is relatively unknown. In many temperate regions, woody browse is an important winter food source for overwintering herbivores, such as hares, moose, and caribou (Bryant et al. 1985, Risenhoover 1989, Den Herder et al. 2004). Studying this relationship has management implications for large ungulates, as changes to stem quantity and quality may have nutritional implications for overwintering animals (Spalinger et al. 2010, Seaton et al. 2011).

Soil nitrogen availability has implications for plant regrowth and chemistry. Increasing available soil nitrogen has been shown to reduce carbon-based plant defenses and increase growth rates (Bryant et al. 1983, Osier and Lindroth 2004, Lindroth et al. 2007). Nitrogen fixation by alder (genus *Alnus*) is an important natural input of nitrogen in ecosystems where it occurs (Nossov et al. 2011). While many studies have examined the effects of artificial

fertilization on plant defensive chemistry and regrowth, relatively few have focused on natural nitrogen sources' role in modulating defensive and growth responses (Lindroth et al. 2007, Stevens et al. 2012).

Previous research has shown that browsing can cause chemical changes within plant tissues. These chemical changes can be induced by a variety of pathways, and the changes vary in magnitude and specificity (Bryant 2003). In the case of dormant winter willow stems, the mechanism is likely to be changes in intra-plant resource competition and availability, rather than a reaction to herbivore saliva or metabolites (Bryant 1987, Bryant 1991). Winter browsing has been shown to reduce bioactivity (and/or concentration) of carbon-based toxins in many woody plants (Veraart et al. 2006, Lindroth et al. 2007).

Salix alaxensis (feltleaf willow) is an extremely important winter browse species for Alaskan moose (Risenhoover 1989). Its leaves and stems are also consumed by hares and caribou (Bryant 2003, Mcart et al. 2009). This willow species is widespread across interior Alaska, and is a common plant species in riparian and early successional habitats (Densmore et al. 1987, Kielland and Osborne 1998, Bowyer and Neville 2003). Despite its importance to wildlife managers, the response of feltleaf willow to winter browsing under natural conditions remains an open question (Risenhoover 1989, Bryant 2003). Feltleaf willows frequently grow in association with alder, which slowly outcompetes willows as ecosystem succession occurs (Wolff and Zasada 1979, Bryant 1987). Feltleaf willows produce condensed tannins as its primary chemical defense, and it has been shown to exhibit changes in tannin activity after browsing and in response to fertilization (Bryant 1987, Bowyer and Neville 2003, Bryant 2003).

The objective of this study was to examine the effects of winter stem browsing by moose, as well as soil nitrogen concentration, on the regrowth, nitrogen concentration, and defensive

chemistry of feltleaf willow leaves and stems produced in the following growing season. I predicted that increasing offtake by moose would result in lower amounts of stem biomass produced the following year, and lower condensed tannin activity in stems and leaves. I also predicted that increased soil nitrogen would correlate with increased stem production, as well as lower tannin activity in stems and leaves. Because moose density is high in the study area, at 2 moose/km^2 in the 2013 fall survey preceding my study (ADFG 2014), and since this confers high browsing pressure on the available forage (Seaton 2002), I expected that feltleaf willows in this location could be browsed to an extent that may reduce their ability to grow stem biomass in the following season.

To evaluate these hypotheses, 2013 stem biomass production and winter offtake by moose were measured across a naturally-occurring range of browsing intensity and alder density during February-March 2014. The following summer, leaves from the same willows were collected and analyzed for nitrogen concentration, ^{15}N natural abundance, and tannin activity. I also collected soil samples across the study sites, which was analyzed for total nitrogen. During fall and early winter 2014, new stem biomass production was measured on the same willows, and stems were analyzed for nitrogen concentration and tannin activity.

METHODS

Study area and site selection

During late February 2014, sixteen feltleaf willow stands were selected on a ~30 km stretch of the Tanana River between Fairbanks and Nenana, Alaska (Figure 1). Potential sites were identified using satellite imagery from Google Earth. The Tanana River is a braided glacial stream, and study sites were selected on point bars, islands and oxbow sloughs within 100m of a

major river channel. The selected stands all contained mature, well-developed feltleaf willow plants ranging from one to three meters in height. Eight sites had thinleaf alder (*Alnus incana ssp. tenuifolia)* interspersed between willow plants, and eight sites had no alders present. Alder stem densities were visually estimated to be between 2,000 and 6,000 stems per hectare using 2012 satellite imagery. This reflects the stem densities typical of a stand where alder are becoming established, and approximately 10,000 alder stems per hectare can be considered dominated by alder (Nossov et al. 2011). High density alder stands were avoided, because they are atypical of areas containing feltleaf willows, and also because shading has been demonstrated to decrease tannin activity in feltleaf willow stems and leaves, which could confound the effects of alder-derived nitrogen in the soil (Bryant 1987).

Sampling design

At each study site, 10 feltleaf willow plants were sampled randomly along an approximately 100 m transect. The transect azimuth was selected haphazardly. Plants were defined as tightly grouped ramet clusters distinctly separated at snow level from adjacent ramet clusters by a minimum of one meter. Riipi et al. showed that in birch, when there is a response to vertebrate browsing, it tends to be restricted to the ramet that receives the damage (Riipi et al. 2005). Knowing this, one representative ramet was haphazardly selected from each plant. These ramets were marked with metal tags and unique identifying numbers. Senescent and obviously diseased plants were avoided, as were those that showed the loss of major branches, which makes accurately assessing browsing history impossible.

After selecting and tagging study ramets (hereafter referred to as plants) I counted the total number of CAG shoots above snow level (approximately 60cm), the total number of old,

dead shoots, and the number of those dead shoots which showed signs of browsing. These parameters are frequently used to assess browsing history of current and previous years (Stolter et al. 2005). For each live CAG shoot, I then measured stem diameter at the stem's base just above the bud scar, and the diameter at bite-point if the stem had been browsed.

I used the diameter at the bud scar of all CAG stems on a plant to calculate CAG production for the 2013 growing season based on published diameter - biomass regressions developed in interior Alaska (Kielland and Osborne 1998). The same relationship was used to calculate the biomass removal using the bite-point diameter of each browsed stem. Proportional biomass offtake for each plant was calculated using as the ratio of the stem biomass consumed by moose to the stem biomass produced by the plant during the previous summer (Eq. 1).

Equation 1: $$\frac{\sum(\text{Biomass Consumed From Each CAG Stem})}{\sum(\text{Biomass of Each CAG Stem})}$$

During the third week of July 2014, foliage samples were collected from the surviving study plants. From each plant, one leaf was collected from ten CAG stems in the plant crown, for a total of ten leaves. If possible, leaves were only collected from the third leaf position on each stem, in order to control for leaf-position influences on defensive response (Bryant 2003, Kleiner et al. 2003). If the ramet had less than ten crown stems, the third and fourth leaf positions were collected and pooled for the sample. Foliage samples were placed on dry ice for transportation to the lab, where they were lyophilized at -40 °C within 48 hours, and homogenized using a Wiley Mill (FAO/IAEA 2000).

To assess soil nitrogen, 4 cm diameter soil cores were taken to a depth of 5 cm at the base of every study plant, on the same day that leaf samples were collected. Intact leaf litter was

removed before coring. Soil samples were stored on ice in a cooler for transport from the field and dried at 40°C within 24 hours. For each site, a composite sample was made, consisting of 1 g of homogenized soil from every core in that site.

Between early October 2014 (after leaf senescence) and mid-January 2015, 2014 CAG biomass was measured on the surviving study plants. Senescent plants and those suffering from visible disease were not sampled, as they show widely varied tannin activities and nitrogen concentrations (Zhang 2008). None of the study plants experienced visible browsing by hares or major insect damage during the sampling events. The number of CAG stems originating at least 60 cm above ground level on each plant was recorded, and the diameter of those CAG stems measured at the bud scar. 2014 growing season CAG biomass was then calculated using the previously mentioned diameter - biomass relationship.

From each plant, three crown CAG shoots were randomly selected for tannin activity (PPC) and total-nitrogen analysis. Because of the ambient freezing conditions, and the stems being dormant, the samples were transported from the field without special consideration for storage. Sample stems were trimmed to a uniform base diameter of 3.5 mm, which is representative of the diameter preferred by foraging moose (Kielland and Osborne 1998). Samples were stored in the lab at -20°C until stem collections were completed, lyophilized at -40°C, and homogenized using a Wiley Mill.

To determine the nitrogen concentration of soil, foliage, and stems, 0.1 g subsamples were analyzed in a LECO 2000 CNS Analyzer (LECO Instruments, St. Joseph, MI, USA). For soil nitrogen concentration, a single subsample was taken from the composite soil sample for each site. For foliage and stem nitrogen concentration, the subsample was taken from each plant.

^{15}N natural abundance was measured in composite samples of homogenized mid-summer foliage, to establish the potential isotopic differences of the nitrogen sources of plants in alder and non-alder sites. Stable nitrogen isotope ratios in plants are determined by a variety of factors, including mycorrhizal relationships, light availability, and the chemical form of the soil nitrogen (Kielland et al. 1998, Hobbie et al. 2009). ^{15}N was used as a potential indicator of alder-derived nitrogen uptake by feltleaf willows. I expected that ^{15}N would be closer to 0, the value for atmospheric nitrogen, in sites with access to alder-derived soil nitrogen. Analyses were performed by the Alaska Stable Isotope Facility at the University of Alaska Fairbanks's Water and Environmental Research Center. 0.05 g of homogenized material was taken from five plants in each site. These were then pooled at the site level, homogenized, and a 0.01 g subsample analyzed for $\delta^{15}N$. This method utilizes a Costech ESC 4010 elemental analyzer and Thermo Scientific Conflo IV interfaced with a Thermo Scientific DeltaV^{Plus} Mass Spectrometer.

Protein precipitation capacity of leaves and stems was measured using a modified version of Robbins' 1987 methods (Robbins et al. 1987, Allman et al. 2018), adapted to use less sample material and a microplate-compatible absorbance reader. Homogenized sample material was mixed thoroughly before a subsample was taken, to evenly distribute the bark or leaf "felt", which tends to stay at the top of samples. This material tends to contain very low protein concentrations compared to the rest of the stem or leaf (K. Kielland, Personal Observation). 125 mg of ground material was added to 10ml of room-temperature 50% MeOH, soaked for 5 minutes in a 15 mL falcon tube, and sonicated at maximum for 15 minutes. The extraction vessels were then capped to prevent MeOH evaporation and allowed to soak for 30 minutes at room temperature. 2.0 mL of the extraction was moved to a micro-centrifuge tube, and spun at 4600 rpm for 18 minutes (Allman 2014).

After centrifugation, 35 µl of sample supernatant was transferred to a centrifuge-able microplate (each sample in triplicate), and 140 µl of 5 mg/ml BSA in 0.2 M acetic acid/acetate buffer with 0.17 M NaCl was added to each cell. The plate was then centrifuged at 5000 rpm for 15 minutes. While the microplates were spinning, standard solutions were prepared with concentrations of 0.5, 1, 1.5, 2, 3 and 5 µg/ml BSA, in the same 0.2 M acetic acid buffer. After centrifuging, triplicate 5 µl subsamples of plant extract/BSA mixture was transferred to a clear microplate for reading absorbance. Standard solutions of each concentration were also added to each plate in triplicate. 250 µl of Bradford protein assay reagent was added to each cell of the microplate containing a sample or standard, and the plate was capped, vortexed for 30 seconds on low, and allowed to sit at room temperature for 6 minutes. Absorbance was then read in a microplate reader at 595 nm using a BioTek Synergy HT Multi-Detection Microplate Reader (Bradford 1976). The absorbance for each sample and standard was calculated as the average of the triplicates from that sample. Protein concentration in each cell was then calculated from the standard curve, and protein precipitation capacities for the corresponding samples were calculated.

All statistical analyses were performed in the statistical program R Ver 3.2.1 (R Core Team 2016). For all analyses, sites were my experimental unit.

To establish that the offtake I measured was representative of each site, I used bootstrapping on proportional biomass offtake (Canty and Ripley 2016). Each site was bootstrapped with 1000 iterations, and visually compared the bootstrapped means to the mathematical means, confirming overlapping standard errors in each site.

Nitrogen concentration in soil, stems, and leaves were analyzed for differences between alder and non-alder sites using t-tests. Equal variances were checked with F-tests, and Welch t-

tests were used if variances were unequal. Normal distributions were confirmed with a Quantile-Quantile plot; 80% or more Q-Q points falling on the 1:1 line was considered a normal distribution.

The effects of current browsing, historical browsing, and soil nitrogen concentration were considered as predictor variables for three separate Ordinary-Least-Squares (OLS) - fitted linear models predicting biomass regrowth, stem PPC and leaf PPC. Intercepts and slopes varied randomly by alder presence in each model. Predictors were selected using all-subsets protocols in the R statistical *'leaps'* package.

Linear regressions assume normal distributions, equal variance, linearity, and non-co-linearity. Normal distributions were confirmed visually with Quantile-Quantile plots, equal variance with F Tests. Linearity and co-linearity were confirmed visually. All raw data used in my models fit the assumptions of normality and linearity and did not require transformation to fit test assumptions. The *a-priori* α for significance was set at $p \leq 0.05$. Marginal significance was set at $p \leq 0.1$.

RESULTS

Across my study sites, dead browsed stems represented 54% of the standing stem population. The number of dead stems increased with total stem count in both alder and non-alder sites (Figure 2). Whereas historic browsing is frequently an important predictor of response to browsing (Stolter et al. 2013), my all-subsets routine (using the *'leaps'* package) revealed that historical browsing was not a good predictor for regrowth, leaf PPC, or stem PPC in my study sites. As such, I did not include it as an independent variable in any models. In keeping with my hypotheses, I included offtake and soil nitrogen concentration as independent variables in all

three models. The number of dead, browsed stems was not related to offtake during the winter of 2013-14 ($F_{1,14}$=0.12, p=0.73).

The bootstrapped mean proportional offtake was not different from the arithmetic mean offtake in any of the sites, confirmed by overlapping standard errors (Figure 3). The close similarity between bootstrapped and arithmetic means implies that sample offtakes were representative of the populations at each site and is a reasonable approximation of the browsing experienced by willows growing in each site.

Total soil nitrogen concentrations in the upper 5 cm of these young soils were very low (~0.1%) and not significantly different between sites with and without alder ($t_{1,14}$=0.827, P=0.42, Figure 4). Biomass consumption by moose increased as plants produced more biomass, and the relationship was not changed by alder presence. Consumption and production (in grams/plant) followed a second order relationship, with an R^2 of 0.73 ($F_{1,14}$=16.2, p<0.01, Figure 5). Pairwise comparison (function: anova) showed that the addition of the alder presence as a predictor of consumption did not improve the model ($F_{12,14}$=0.50, p=0.62), indicating that the relationship between production and consumption did not differ between alder and non-alder locations.

Plants growing in the absence of alder produced from 23.9 to 36.1 grams of biomass per plant, with an average of 29.3 ± 1.44 g, while plants growing with alder present ranged from 11.9 to 45.1 g/plant with an average biomass of 30.9 ± 4.40 grams (Figure 6). 2013 stem biomass had a linear relationship with 2014 stem biomass produced in 2014 (Figure 7). Because of this relationship, proportional regrowth (grams of biomass produced in 2014 divided by grams of 2013 biomass) was used to model response to browsing. Failure to account for the large differences in initial biomass, and presumed initial differences in internal plant resource pools

also may have obscured any response to browsing intensity relative to plant size (Hochwender et al. 2011, McNickle and Evans 2018).

The range of browsing was very similar between alder and non-alder sites. In non-alder sites, proportional offtake ranged from 0.28 to 0.45, with an average of 0.34 ± 0.028 (Figure 8). In alder sites, offtake ranged from 0.21 to 0.56, and averaged 0.33 ± 0.038 (Figure 8). Proportional regrowth averaged 0.86 in both alder and non-alder sites and was not significantly different between them ($t_{1,13}$=1.76, p=0.42, Figure 9). After pooling alder and non-alder sites, regressing proportional regrowth against proportional offtake showed a positive and marginally significant relationship ($F_{1,13}$=1.35, p=0.03, R^2=0.09). However, this relationship was driven by a single site. When the site was removed from the model, the slope was no longer significant ($F_{1,13}$=0.17, p=0.68, Figure 10).

My linear model predicting proportional regrowth in response to proportional offtake and soil nitrogen concentration was not significant (p=0.55) and had an R^2 of 0.32 (Table 1). In both alder and non-alder sites, regrowth in the year following browsing did not provide evidence that offtake influenced regrowth in a significant fashion (p= 0.40, Table 1). Neither intercept was significant with p=0.81 and p=0.12 in alder and non-alder sites, respectively (Table 1).

Leaf and stem quality both differed between alder and non-alder sites, though the differences were not always statistically significant. Foliar nitrogen concentration was lower in non-alder sites (1.56 ± 0.056%) than in alder sites (1.79 ± 0.042%), and the difference was significant ($t_{1,13}$=3.18, p=0.007, Figure 11). Average stem nitrogen concentration was also lower in non-alder sites, averaging 0.88 ± 0.035% compared to 0.96 ± 0.035% in alder sites, but the difference was not significant ($t_{1,13}$=1.58, P=0.135, Figure 11). Similarly, I found non-significant effects of alder presence on tannin activity (PPC). Foliar PPC was nearly identical in both alder and non-

alder sites, averaging 126 ± 9.84 mg BSA/g and 123 ± 12.8 mg/g in non-alder and alder sites, respectively (Figure 11). Stem PPC was lower in non-alder sites, with an average of 89 ± 10.1 mg BSA/g vs 105 ± 12.1 mg/g in alder sites, though the difference was not significant (Figure 11).

The linear model predicting foliar PPC in response to proportional offtake and soil nitrogen concentration was not significant (p=0.42) and had an R^2 of 0.35 (Table 2). The intercept for non-alder sites was significant (p=0.04), while the alder site intercept was not (p=0.56). Soil nitrogen was a marginally significant, negative predictor of foliar PPC in sites without alder (p=0.09) but was not a significant predictor in sites with alder present. By contrast, stem PPC was negatively associated with proportional offtake and positively associated with soil nitrogen concentrations (p = 0.03, R^2 = 0.66; Table 3). The intercept of the linear model in alder sites was significant and positive (p=0.01), while the intercept in non-alder was marginally significant (p=0.09). Proportional offtake was an insignificant predictor of stem PPC in non-alder sites but had a significant and negative association with PPC in alder sites (p=0.02). Soil nitrogen concentration was positively associated with stem PPC in non-alder sites (p=0.03) but was not a significant predictor in alder sites. Lastly, foliar $\delta^{15}N$ signatures of feltleaf willows did not vary between alder and non-alder sites. Both site types had very similar, and highly depleted isotope values (~ -6‰, Figure 12).

DISCUSSION

In this study, I explored the effects of wintertime moose browsing and soil nitrogen concentration on the stem regrowth and forage quality of feltleaf willows. I expected that winter browsing would decrease stem biomass produced the following growing season and increase the

nutritional quality of that season's stems and leaves; specifically, through reduced PPC. However, the ranges of browsing and soil nitrogen concentrations found in my study sites were not associated with changes in regrowth, but were related to moderate changes in foliar and stem PPC though those changes were inconsistent between alder and non-alder sites (Tables 1-3). Foliar N content was higher in sites with alder present (Figure 11), though this was not associated with higher total soil nitrogen concentration (Figure 4).

I hypothesized that winter browsing would reduce CAG biomass production in the following growing season. I reject this hypothesis, as the slope of the relationship between browsing and regrowth was not different from zero in the presence or absence of alder (Table 1, Figure 10). This indicates that feltleaf willows growing on the Tanana River floodplain can acquire enough resources to compensate aboveground for winter browsing, even when more than 40% of CAG stem biomass has been removed. I also hypothesized that total soil nitrogen would modify the regrowth response, but this parameter also proved to be an inconsistent predictor of regrowth (Table 1). Regrowth after winter browsing in *Salix* is highly variable, but my results are consistent with other research, where winter high levels of winter offtake can have a negligible effect on next-year aboveground biomass production in willows (Guillet et al. 2006).

I initially expected that browsing would reduce PPC in stems and leaves. This hypothesis was based on previous research in this ecosystem on the effect of whole-plant carbon/nitrogen ratios on secondary defensive chemistry, and the existence of "escape responses" where plants that experience browsing respond by growing long shoots to escape herbivory (Bryant et al. 1983, McNaughton 1983, Bryant 1987, Bryant 1991). However, I found that the magnitude of browsing was not consistently related to PPC in stems or leaves (Table 2, Table 3). Stems in

alder sites were the only group that acted as hypothesized, with lower PPC values in plants that experienced higher offtake (Table 3).

In sites with alder present, stem PPC was 18% higher than in sites without alder, though the variation was high in both groups and the difference was not significant (Figure 11). As plants at non-alder sites did not experience higher browsing pressure or have higher soil nitrogen concentrations, other variables such differences in root:shoot ratio and increased intra-plant resources may have come into play (Hochwender et al. 2011). Many of the individual willows growing in sites with alder were generally older and more structurally complex with more ramets and total stems (Personal Observation) and presumably have larger root systems and larger pre-browsing nitrogen and carbon pools which could allow for both compensatory growth and defense (Hochwender et al. 2011). Veraart et al. (2006) found that nitrogen and total phenolic concentrations of willow bark collected in November did not differ between clipped and un-clipped willow plants, despite significant differences in leaf nutrition during the summer. They suggest that the post-browsing growth phase with lower carbon-based defenses is isolated to the early growing season, which can lead to improved leaf nutrition in browsed willows, whereas the defensive chemistry of stems approaches that of unbrowsed stems later in the growing season. This may also be the case in feltleaf willows recovering from winter browsing by moose. In any case, differences in summer stem chemistry are largely unimportant to moose, as they consume stems predominantly during the winter months (Risenhoover 1989).

Mid-summer foliar PPC was not lower in plants that experienced higher levels of offtake (Table 2), which is in accordance with previous research (Bryant 2003). Because moose do not appear to extensively leaf-strip feltleaf willows in this region (Personal Observation), foliar PPC may not have major nutritional ramifications for moose along the Tanana River. However, in

other parts of Alaska, these leaves are an important part of the moose diet (Mcart et al. 2009). If feltleaf willow in those areas has a similar lack of response in their mid-summer foliage to browsing, it would be beneficial to those moose populations. These results also bode well for other vertebrate herbivores that feed heavily on feltleaf willow leaves during the summer, which includes interior Alaskan caribou and snowshoe hares (Boertje 1984, Bryant 1987, 2003). It should be noted that many herbivores consume willow leaves during spring and early summer during the birthing season, and these results cannot be extrapolated to those time-periods, as the nitrogen concentration and tannin activities of feltleaf willow both vary significantly over the course of the growing season (Kielland et al. 1998, Bryant 2003, McArt et al 2009).

While my study did not find consistent relationships between regrowth or PPC and winter browsing, there are several issues with my sampling protocol that could have caused me to miss a potential response. I only sampled a single ramet out of each cluster, and sites where alders are present tend to have older, more mature willow plants with more ramets (Personal Observation). Older, more complex plants introduce two potential problems: 1) the ramet may not accurately represent the browsing experienced by the entire plant, and 2) more mature plants will have a larger root system in place when spring arrives, and more resources to put towards regrowth.

Another potential problem in my sampling protocol is the exclusion of stands with higher alder density. My results showed that total soil nitrogen concentration was not significantly higher in sites where alders are present (Figure 4). Across a range of stand densities of 10,000 to nearly 30,000 alder stems per/ha, Nossov et al. (2011) found a linear increase in total soil nitrogen concentration. However, my study plots were well below these stem densities. This was necessary to avoid confounding shading effects on carbon allocation, but may be worth further investigation (Bryant 1987). Also, the $\delta^{15}N$ signatures of willow foliage in mid-season

was not different regardless of alder presence (Figure 12). The lack of different $\delta^{15}N$ signatures shows that my original expectation that alder-derived nitrogen would produce a significant difference in nitrogen isotope ratios was incorrect. It could be the case that plants were drawing from abundant sources of N such as nitrate in hyporheic water (Clilverd et al. 2008, Koyama and Kielland 2011). It is also possible that alder-derived nitrogen undergoes fractionation events before being incorporated into willow leaves that make it indistinguishable from other sources of nitrogen (Hobbie et al. 2009). This would be consistent with other research that found no difference in $\delta^{15}N$ between poplars growing in the presence of alder or in a monoculture (Kurdali et al. 1990).

Unusual weather conditions may also have contributed to my results. For example, the months of June and July 2014 were the wettest and second wettest on record (ACRC 2014), and 11 of my 16 plots had standing water present when I collected soil samples. Standing water was present in some form or another, depending on the site, for all of June, and most of July. This is highly unusual for this ecosystem, and I cannot comment on the effects this may have had in altering soil nitrogen concentration or the $\delta^{15}N$ signature of the soil. However, Tanana River water is known to contain high concentrations of nitrate during the summer (85 µg/l; Clilverd et al. 2008), which may have acted as an abundant, direct, source of nitrogen for the plants (Clilverd et al. 2008). This may be particularly important in the early-succession, alder-free stands I sampled, which only accrue 26% of their annual nitrogen requirements from soil N mineralization (Lisuzzo et al. 2008), but could also impact sites with alder present, which contained only low alder densities (Lisuzzo et al. 2008).

My results yield some food for thought regarding moose-willow interactions in riparian ecosystems. Insofar as moose browsing did not reduce the magnitude of subsequent stem

biomass production even at high offtake rates (>40%), and further that winter browsing has negligible effects on forage quality in terms of nitrogen concentrations and tannin activity in leaves and stems, it appears that feltleaf willows on this floodplain are very resilient to high browsing pressure. Future research should be directed at other effects, such as flower inhibition and reduced below ground allocation to gain a more complete understanding of the impacts of moose browsing on feltleaf willow.

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Table 1. Regression results for linear model predicting regrowth response to proportional biomass offtake by moose and soil nitrogen concentration. Intercepts and slopes vary by alder presence. Model RSE=0.254, R^2=0.32, $F_{6,10}$=0.853, p=0.545.

Variable	Value	SE	P-Value
Intercept: Non-Alder	1.44	0.850	0.124
Intercept: Alder	1.82	1.24	0.805
Slope: Offtake, Non-Alder	-0.222	1.21	0.858
Slope: Offtake, Alder	0.878	0.987	0.397
Slope: Non-Alder, Soil Nitrogen	-0.515	0.765	0.518
Slope: Alder Soil, Nitrogen	-1.32	1.11	0.266

Table 2. Regression results for linear model predicting foliar PPC response to proportional biomass offtake by moose and soil nitrogen concentration. Intercepts and slopes vary by alder presence. Model RSE=0.031, R^2=0.35, $F_{6,10}$=1.09, p=0.42.

Variable	Value	SE	P-Value
Intercept: Non-Alder	0.244	0.103	0.039*
Intercept: Alder	0.348	0.140	0.564
Slope: Offtake, Non-Alder	-0.105	0.146	0.491
Slope: Offtake, Alder	-0.005	0.117	0.963
Slope: Soil Nitrogen, Non-Alder	-0.085	0.093	0.382
Slope: Soil Nitrogen, Alder	-0.236	0.126	0.091

Table 3. Regression results for linear model predicting stem PPC response to offtake by moose and soil nitrogen concentration. Intercepts and slopes vary by alder presence. Model RSE=0.023, R^2=0.66, $F_{6,10}$=3.84, p=0.033.

Variable	Value	SE	P-Value
Intercept: Non-Alder	-0.144	0.076	0.088
Intercept: Alder	0.239	0.103	0.014*
Slope: Offtake, Non-Alder	0.191	0.108	0.109
Slope: Offtake, Alder	-0.233	0.087	0.023*
Slope: Soil Nitrogen, Non-Alder	0.172	0.069	0.031*
Slope: Soil Nitrogen, Alder	0.058	0.094	0.552

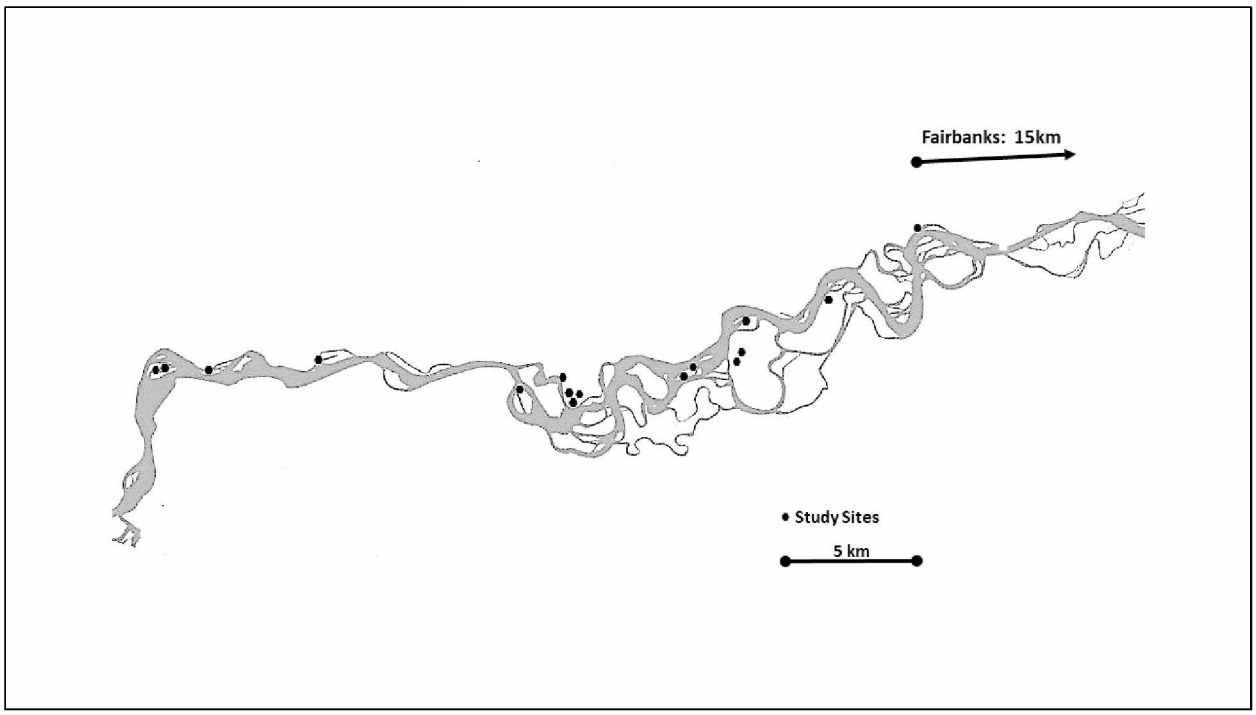

Figure 1. Map of study area on the Tanana River floodplain.

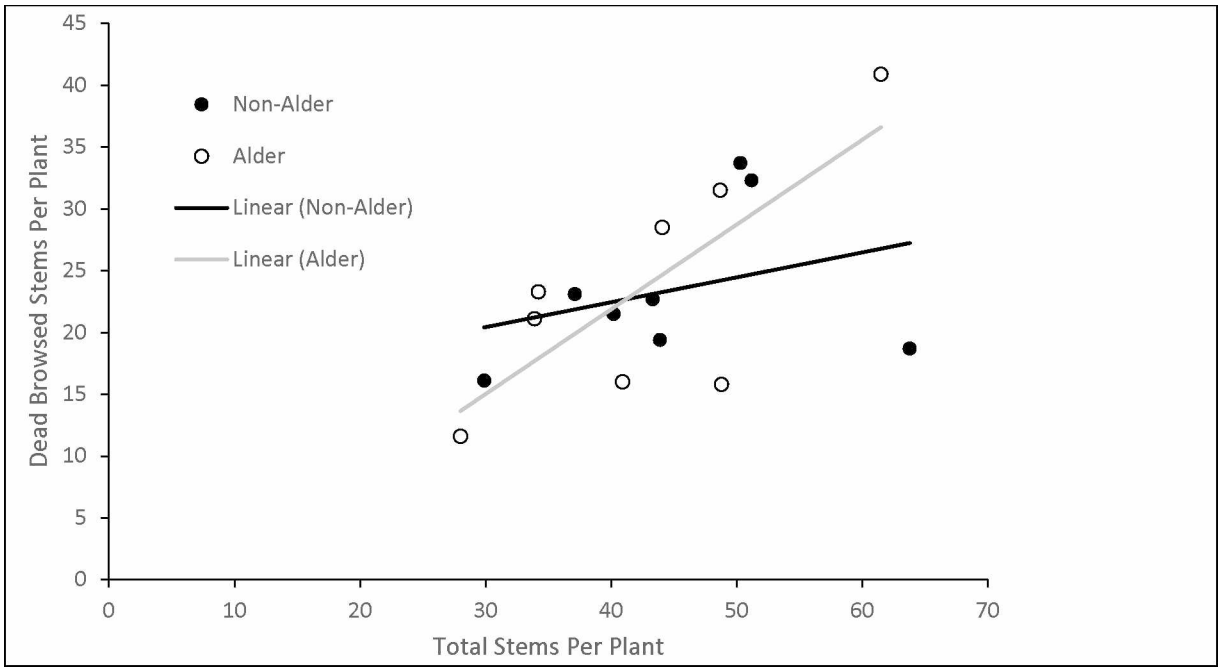

Figure 2. Relationship between total and browsed dead stem count (site averages) of feltleaf willows growing on the Tanana River floodplain.

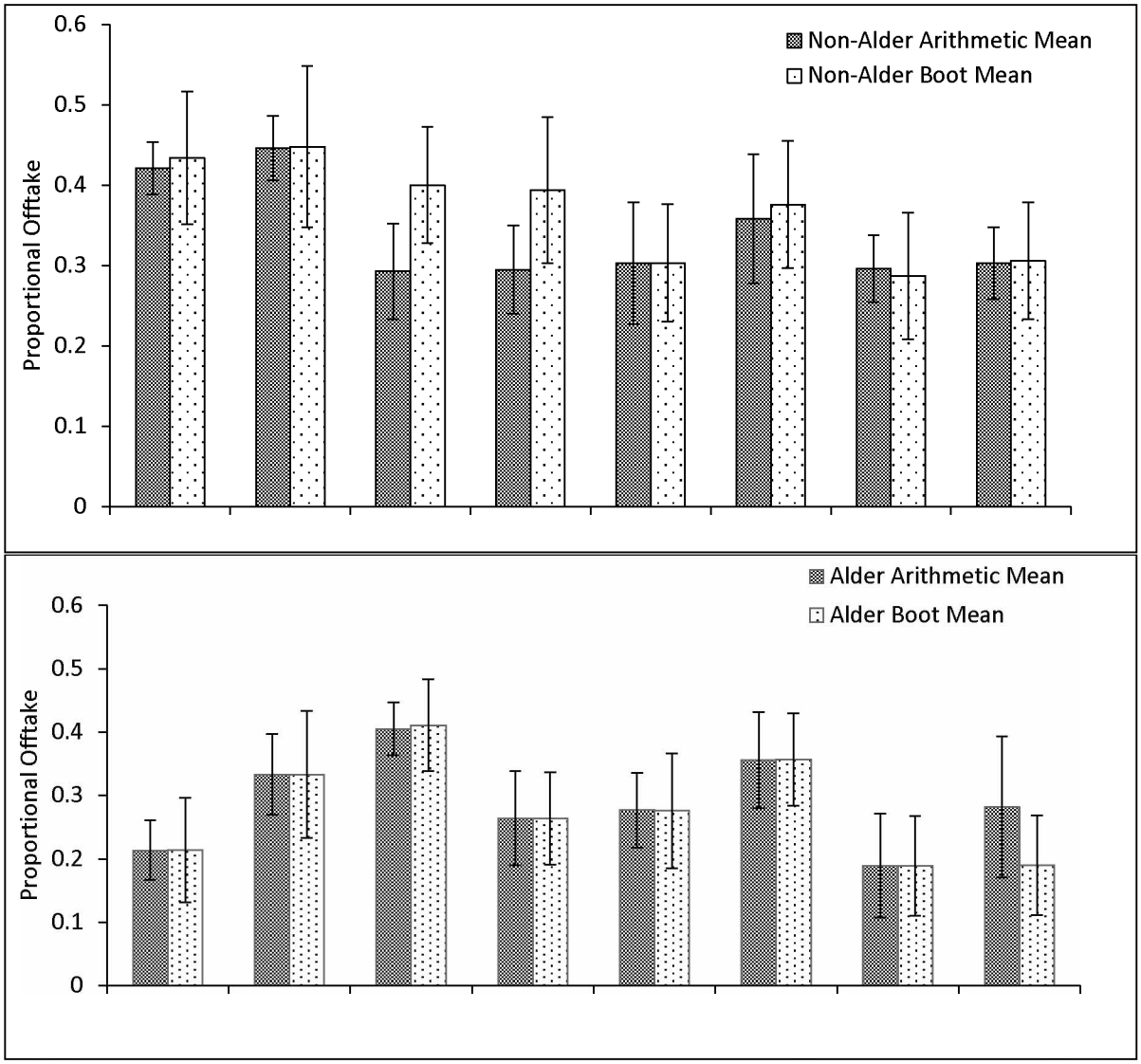

Figure 3. Bootstrapped and arithmetic mean of offtake by moose consuming feltleaf willows on the Tanana River floodplain in sites with alder absent (top) and alder present (bottom). Site mean ± SE, n=10. Arithmetic mean and SE in dark bars, bootstrapped means and SE in light bars. Sites are arranged by their location on the Tanana River relative to Fairbanks, from upstream to downstream, in the presence and absence of alder.

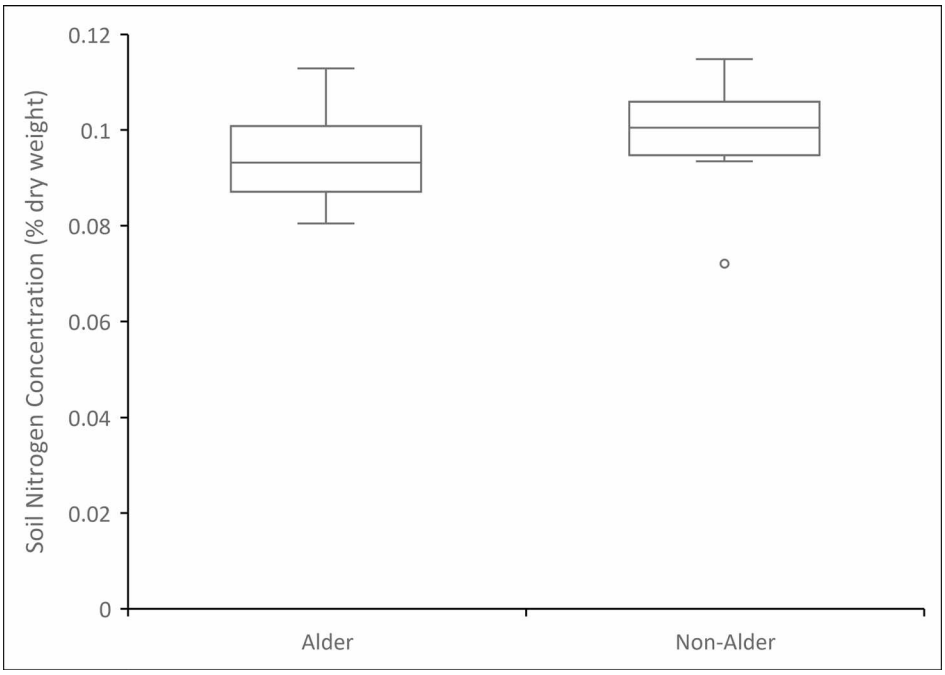

Figure 4. Soil nitrogen concentration of composite soil subsamples at study sites in the presence and absence of alder. Site averages are shown. Box shows 1st and 3rd quartiles, median centerlines, whisker min and max, dot outliers, n=16, 8 per group.

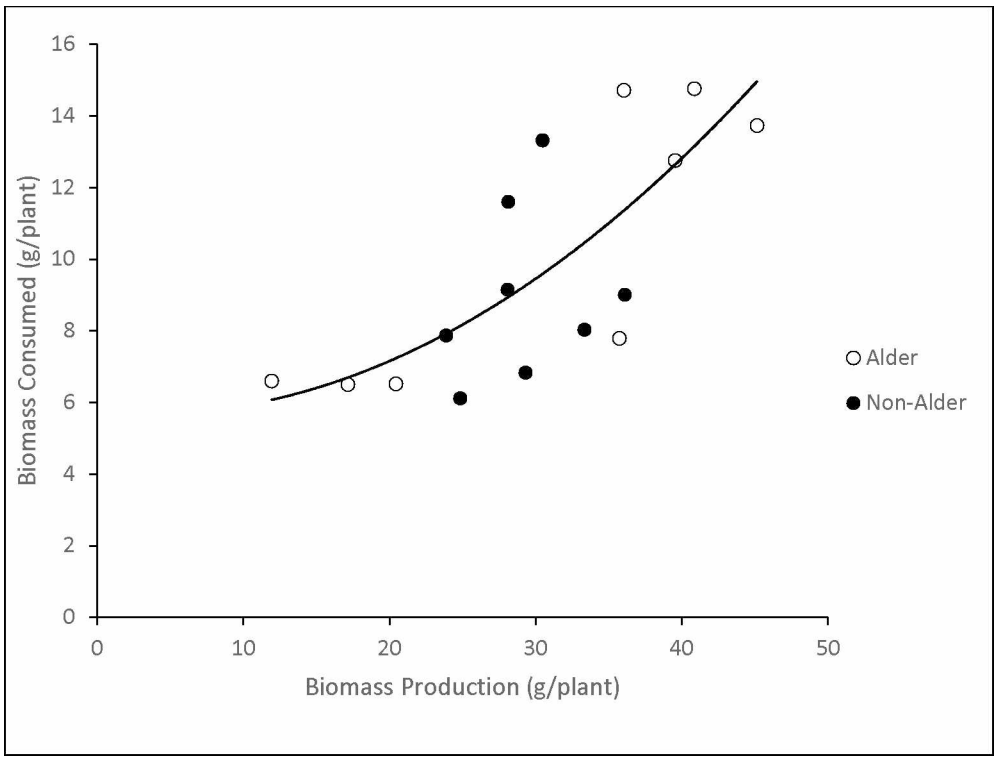

Figure 5. Biomass consumption vs biomass production of feltleaf willows on the Tanana River floodplain. Site arithmetic means are shown.

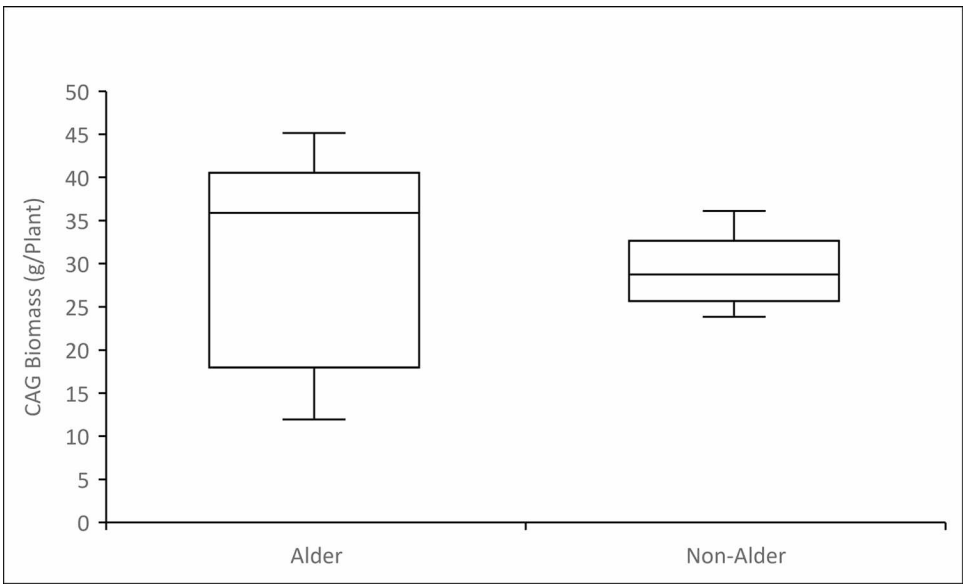

Figure 6. Current annual growth biomass (g/plant) of feltleaf willows at beginning of study. Site averages are shown. Box shows 1st and 3rd quartiles, median centerlines, whisker min and max, n=16, 8 per group.

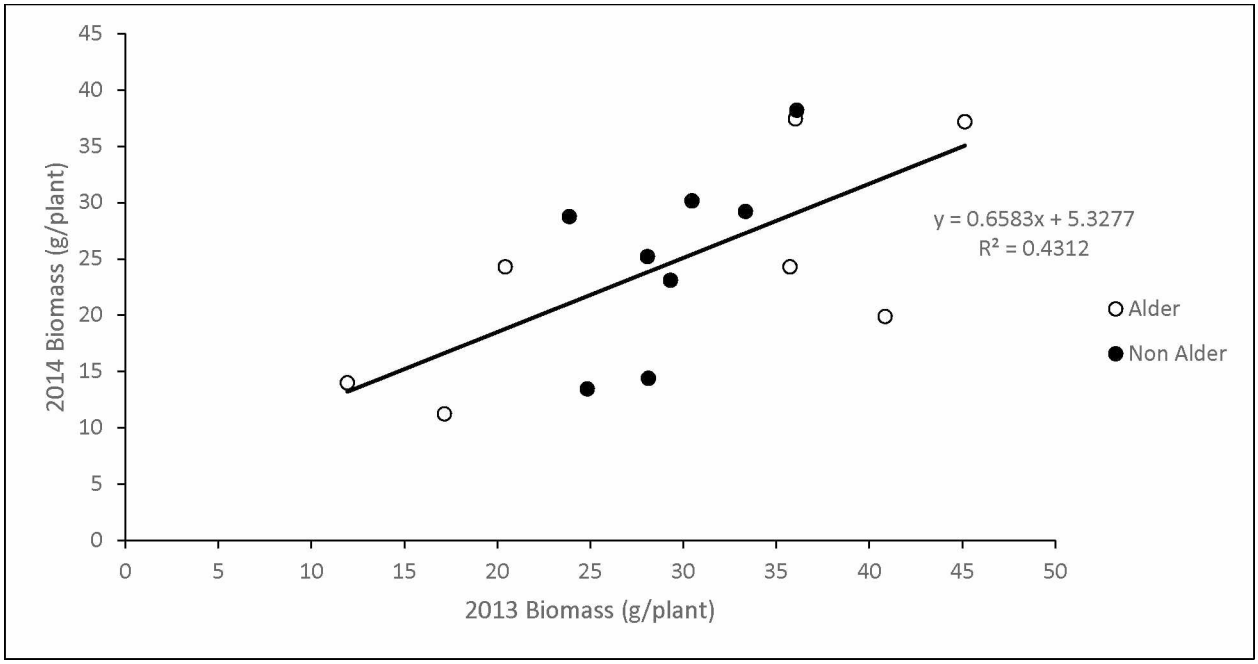

Figure 7. 2013 biomass vs 2014 biomass produced by feltleaf willows on the Tanana River floodplain. Site arithmetic averages are shown.

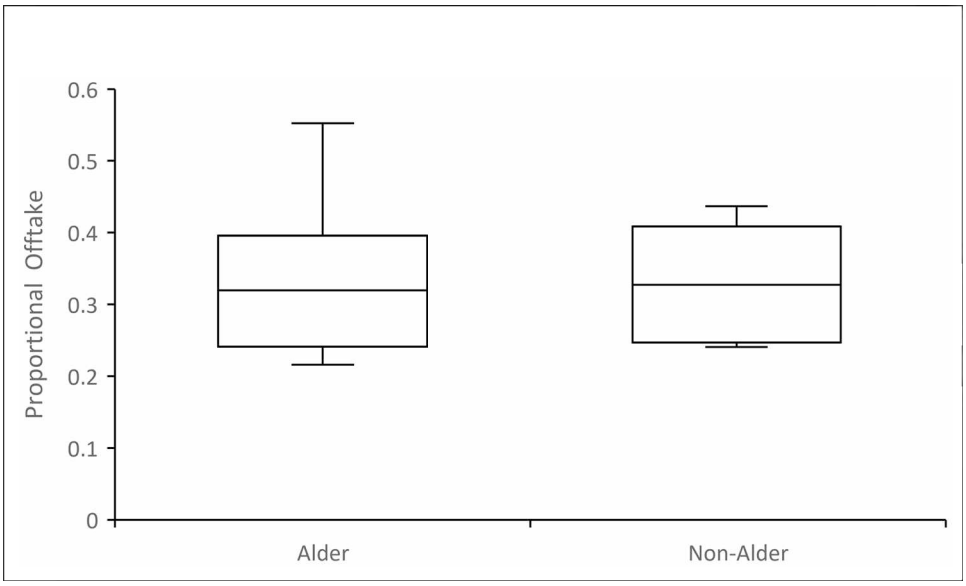

Figure 8. Proportional offtake by moose foraging on feltleaf willows in sites with and without alder on the Tanana River floodplain. Site averages are shown. Box shows 1st and 3rd quartiles, median centerlines, whisker min and max, n=16, 8 per group.

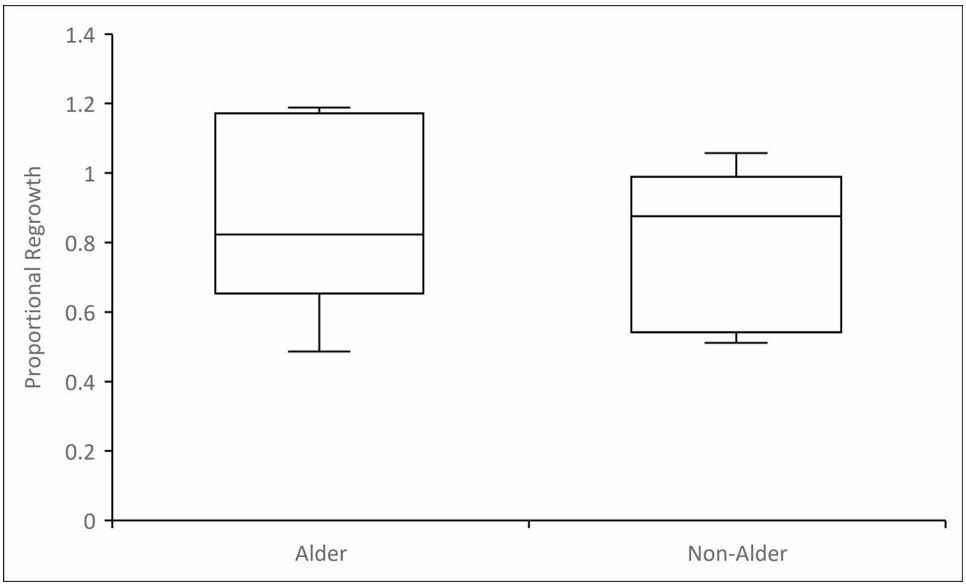

Figure 9. Proportional regrowth of feltleaf willows on the Tanana River floodplain in the presence and absence of alder. Site averages are shown. Box shows 1st and 3rd quartiles, median centerlines, whisker min and max, n=16, 8 per group.

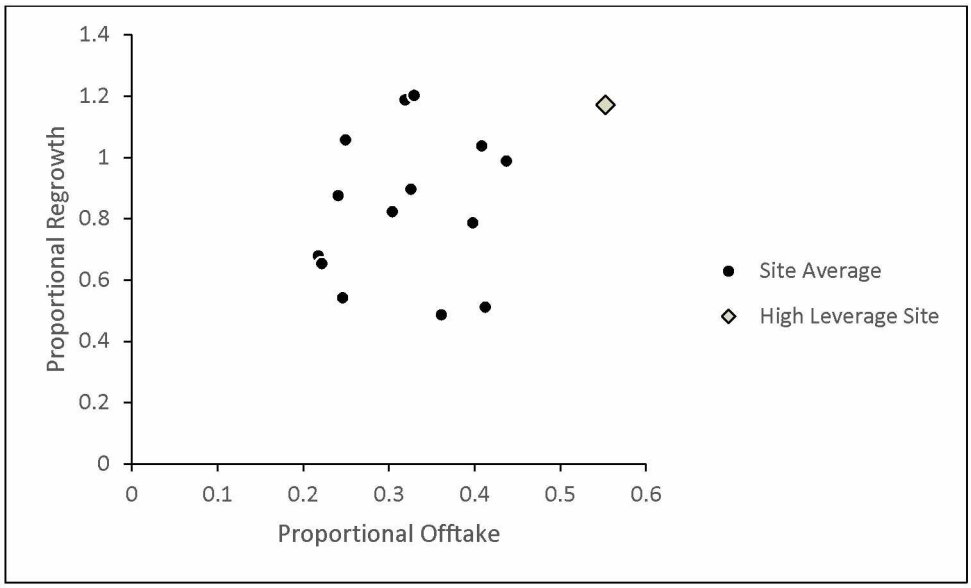

Figure 10. Proportional regrowth in response to offtake by feltleaf willows growing on the Tanana River floodplain. Data are pooled from alder and non-alder sites. With high leverage site excluded, the regression not significant ($F_{1,13}$=0.17, p=0.68, R^2=0.01).

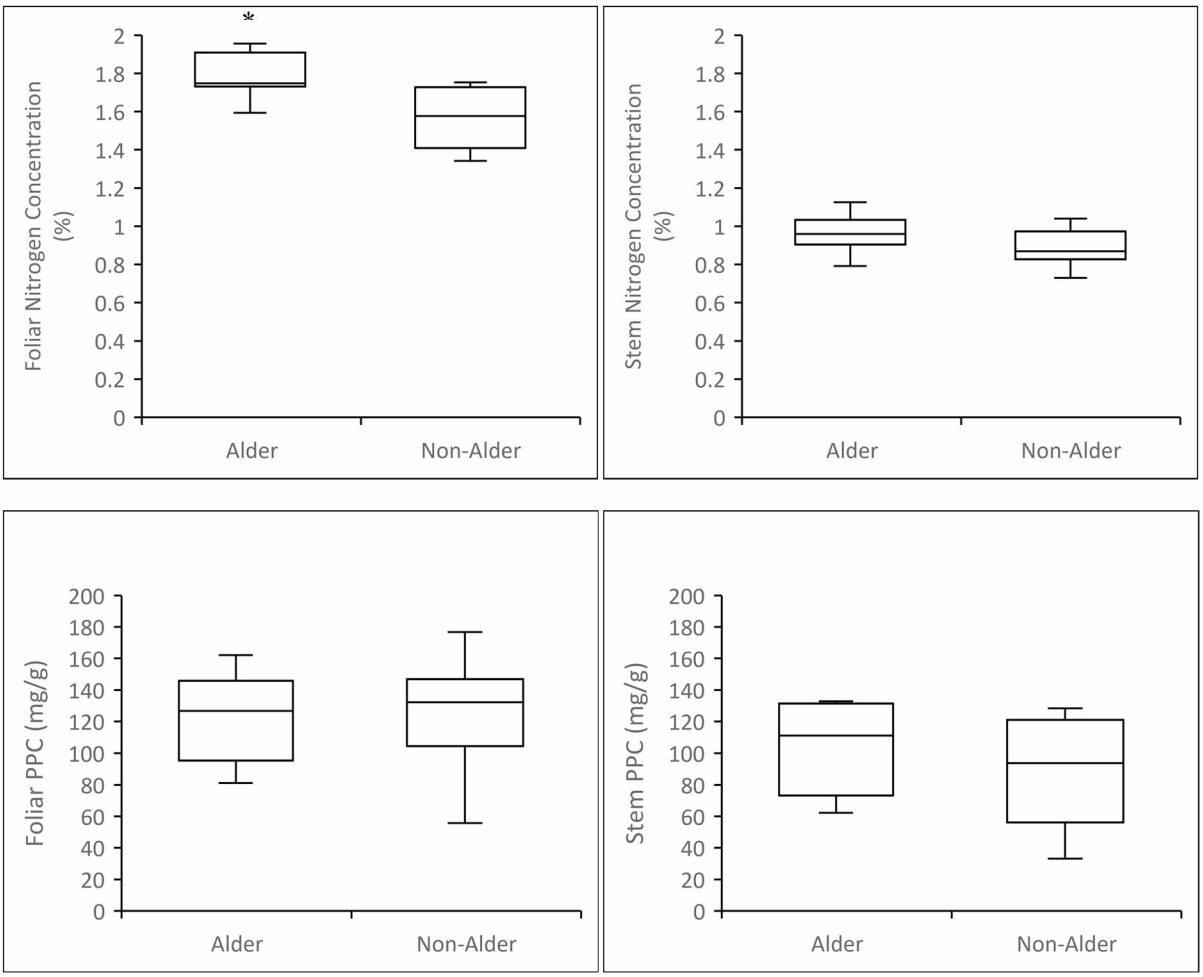

Figure 11. Nitrogen concentration (% dry mass) and PPC (mg/g plant material) of feltleaf willow foliage and stems growing in the presence and absence of alder. Boxes shows 1st and 3rd quartiles, median centerlines, whisker min and max, n=16, 8 per group.

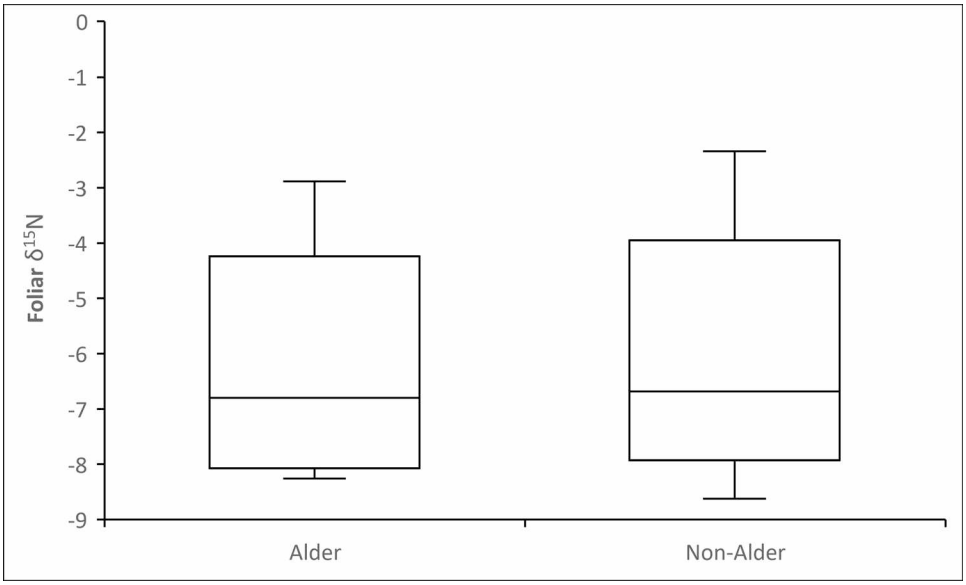

Figure 12. $\delta^{15}N$ of feltleaf willow foliage composite subsamples collected in summer 2014 on the Tanana River floodplain. Box shows 1st and 3rd quartiles, median centerlines, whisker min and max, n=8.

GENERAL CONCLUSION

The results of this study provide several interesting pieces of information about feltleaf willows growing on the Tanana River floodplain. Felfleaf willows in this ecosystem were remarkably resilient to high browsing pressure, and did not exhibit a trend of reduced stem biomass production across the range of offtake found in my study plots. Browsing also was not consistently related to changes in stem and leaf chemistry. The relationships between defensive chemistry and regrowth to soil nitrogen concentration were also variable. These results suggest that plants growing in this ecosystem have abundant resources and can acquire sufficient nutrients to recover from high levels of winter offtake.

I observed similar physical and chemical characteristics in feltleaf willows growing in the presence and absence of alder. Despite differences in the distributions of biomass between alder and non-alder sites, average biomass, proportional offtake, and proportional regrowth were all very similar (Figure 6, Figure 8, Figure 9). Foliar nitrogen concentrations were significantly higher in sites with alder (Figure 11). Total soil nitrogen, my proxy for alder-derived nitrogen presence, was not different between sites with and without alder (Figure 4). The higher foliar nitrogen concentration may indicate a larger nitrogen source in sites with alder that is not evident from total soil N, but may be obscured by rapid turnover in those locations. While alder has frequently been associated with greater soil nitrogen concentrations, my sites may have been at of the wrong successional stage to detect any difference (Tarrant and Miller 1963, Van Cleve et al. 1971).

Physical and chemical relationships with browsing and soil nitrogen concentration did not show consistent trends across site types. I predicted that increased offtake would be related to a decrease in stem biomass production, and that increased soil nitrogen concentration would

increase production. However, there were no significant relationships between either offtake or soil nitrogen concentration and proportional regrowth (Table 1). Response to stem browsing in family *Salicaceae* is highly variable, and previous research has shown variable responses in stem biomass production to stem browsing (Peinetti et al. 2001, Keefover-Ring et al. 2016). I also predicted that increasing soil nitrogen concentration would be associated with increased regrowth and decreased PPC. By contrast, the only significant relationship I discovered between soil nitrogen and PPC was a positive relationship in stem PPC to increasing soil nitrogen in sites without alder (Table 1). The only group that behaved as predicted was stems of plants growing in sites with alder, where stem PPC declined with increased offtake (Table 2).

Previous work had found changes in willow foliar and stem defensive chemistry in response to winter browsing, however, those same changes are sometimes fleeting, and may only be apparent early in the growing season, with defensive chemistry approaching that of unbrowsed tissues by the end of the growing season (Bryant 2003, Veraart et al. 2006). It may be that my sampling events were simply too late in the year to measure any difference in defensive chemistry, given that my first foliage collections took place in July. However, because the focus of my study was to assess the impact of browsing on dormant winter stems that will be available the following winter, these results are still valuable from a wildlife management perspective.

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